

A STUDY OF SOME SMUTS OF *ECHINOCHLOA* SPP.

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(Plates XXIII–XXVIII)

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Synopsis

The structure and development of sori of *Ustilago tricophora* on *Echinochloa* spp. have been described. There is no essential difference between the sori formed in floral parts and those occurring in vegetative parts of the host. Sporogenous hyphae growing from the region of the columella form spores progressively, the first formed spores being those just beneath the soral covering. The latter is composed of a fungal sheath overlain by a hispid covering of host origin. A study of the development of the smut in both inoculated plants and naturally infected plants and a scrutiny of herbarium specimens of smut of *Echinochloa* from many parts of the world have shown that the smut which occurs in floral organs or in vegetative parts and which is ornamented by spines is *Ustilago tricophora* (Link) Kunze. The names *Ustilago sphaerogena*, *U. crus-galli*, *U. globigena* and *U. panici-frumentacei* have been applied to smuts which have now been shown to be indistinguishable from *U. tricophora*.

INTRODUCTION

Smut fungi are known as parasites of species of *Echinochloa* (Gramineae) in many parts of the world. Seven species of *Ustilago* have been described from these grasses, the earliest being an ovary smut, *Ustilago tricophora* (Link) Kunze on *Echinochloa colonum* from Egypt. Apart from its formal description by Kunze (1830) almost nothing has been written about that smut. Another ovary smut, *Ustilago sphaerogena* Burrill, described in 1888, has been studied briefly by Fischer (1953) and is recorded from New South Wales (Anon., 1958). *Ustilago crus-galli* Tracy & Earle was described in 1895 as affecting both the inflorescence and the vegetative parts, and McAlpine (1910) has recorded the occurrence of this smut in Australia. *Ustilago panici-frumentacei* Bref., *U. globigena* Speg., *U. paradoxa* Syd. & Butl. and *U. holubii* Syd. which were described in 1895, 1898, 1911 and 1935 respectively have a number of points in common with the smuts described in earlier years, though the last mentioned entirely destroys the panicle while the others form sori in only some of the spikelets in an inflorescence. Fullerton (1966) in a short report of his studies of soral development of a smut in parts of the inflorescence of *Echinochloa colonum* referred that smut to *Ustilago sphaerogena* Burrill, using the work of Fischer (1953) as a guide to its identity. Fullerton found that galls similar to those in the floral parts were sometimes developed on the vegetative parts of plants that had been inoculated with the ovary smut known to him as *U. sphaerogena*. He also pointed out that the differences between *U. sphaerogena* and *U. crus-galli* were differences of degree rather than of kind and foreshadowed a taxonomic investigation of smuts of *Echinochloa*.

Species of *Ustilago* on *Echinochloa* have been studied and the results are reported in this paper. An account is given of the development and structure of sori and of sporogenesis in both reproductive and vegetative parts of the host. Using these and other data, a taxonomic revision of certain smuts has been made.

It will be convenient in the following discussions of the smuts of *Echinochloa* to refer to some of the specimens in terms related to the type of sorus, i.e., the symptoms, induced in the host and to the form of the spines developed on the spore wall. Sori developed separately from various organs of the floret are referred to as the *U. sphaerogena* form and sori on vegetative parts or developed in the inflorescence as galls not specific to particular organs are termed the *U. crus-galli* form. Spines on the spores are referred to as being of the *U. sphaerogena* form or the *U. crus-galli* form when they resemble the spines occurring on the spores in the type material of these species.

MATERIALS AND METHODS

Specimens of *Echinochloa colonum* infected by a smut of the *Ustilago sphaerogena* form were collected in the Rosewood district of south-eastern Queensland during the late summer and autumn months (Feb.-May) of 1964 and 1965. In May, 1966, specimens were collected from the Meandarra and Goondiwindi districts of Queensland. Some of the material from these collections was dried, the remainder being preserved in Formalin Acetic Alcohol (FAA).

In October, 1964 and September, 1965, seeds of *Echinochloa colonum* were dusted with spores of the *U. sphaerogena* form of smut and planted in a garden plot at St. Lucia. A number of these plants developed sori. A detailed study was made of the morphological and anatomical features of the various soral types represented. Sori in different stages of development were fixed in FAA. Material to be sectioned was dehydrated in a tertiary butyl alcohol series and embedded in paraffin wax of MP 54-56° C. Blocks were serially sectioned at 10 μ , sori being cut both longitudinally and transversely. Two methods of staining were used, Safranin-Fast Green (Johansen, 1940) and Periodic acid-Schiff's Reagent (Conn, 1960) with a Fast Green counterstain. Both methods were satisfactory for differentiating mycelium and host tissues.

Details of spore formation were elucidated by examining the appropriate parts of the serial sections by phase contrast microscopy. Some useful supplementary information was obtained by hand teasing young sori, treating the sporogenous hyphae with concentrated potassium hydroxide to remove gelatinous material, mounting in clear lactophenol and examining by phase contrast microscopy.

For the taxonomic work on the smuts of *Echinochloa* spp. specimens from a number of herbaria were obtained. These were supplemented by material collected from plants in the field and smut-inoculated plants grown in garden plots in the grounds of the University of Queensland at St. Lucia, Brisbane. Spores for microscopic examination were mounted in Shear's mounting fluid (Graham, 1959). Herbaria from which specimens have been made available are as follows: United States National Fungus Collection, U.S.D.A., Beltsville (BPI); National Herbarium, Department of Agricultural Technical Services, Republic of South Africa (PRE), New South Wales Department of Agriculture, Rydalmere (DAR); Instituto de Botanica C. Spegazzini, La Plata, Argentina (LPS), Commonwealth Mycological Institute (IMI); Department of Botany, University of Queensland (BRIU).

MORPHOLOGY OF SORI

The sori on *Echinochloa* spp. are readily detected because parts of the plants where the fungus sporulates increase in size and bear numerous hairs on the surface. The hirsute nature of the soral covering was noted by Kunze (1830) when he described *Ustilago tricophora*, and similar observations have been made Burrill (1888), Tracy and Earle (1895) and

Mundkur (1943) in their descriptions of various species of *Ustilago* which occur on *Echinochloa* spp. Magnus (1896) in describing the smut galls on *E. crus-galli* referred to the presence of a sheath of fungal hyphae underlying the host tissue that covered the swollen areas. McAlpine (1910) also has commented briefly on the structure of the galls studied by Magnus but added no new information of any significance. The development of sori in vestigial ovaries in staminate spikelets of *Buchloe dactyloides* infected by *Tilletia buchloeana* was reported by Norton (1896). Hansing and Lefebvre (1941) described sori of *Sphacelotheca sorghi* and *S. cruenta* where stamens as well as the ovaries had been transformed by the smut and they referred also to sori developing in the rudimentary ovaries of staminate spikelets of *Andropogon furcatus* infected by *Sorosporium everhartii*. Fullerton (1966) has shown that smuts of *Echinochloa* spp. which could be referred to *Ustilago sphaerogena* were not confined to the ovary but spored in various organs of the floret and in stems and leaves.

STRUCTURE OF SORI IN INFLORESCENCES

Since the form of the sori is influenced by the structure of the organs or parts in which sporulation occurs, the morphology of floral parts of *Echinochloa colonum* is described briefly here. Spikelets consist of two florets, the lower floret being sterile and the upper one fertile. The sterile floret has a lemma and a membranous palea only. The fertile floret has a lemma which is hard and shiny with inrolled margins. With a thin palea it encloses the floral organs. Small unicellular hairs occur on the outer surfaces of the glumes, the sterile lemma and the pedicel of the spikelet below the glumes. On the axes of the panicles are long, pointed, unicellular hairs which arise in the epidermis.

The ovary is smooth, sub-globose and glistening white, about 0.5 mm. long, with two styles and feathery stigmas. The ovary wall surrounding the ovule is two cells thick. The ovule is anatropous with two integuments surrounding the nucellus. There are three stamens and two lodicules. The latter are flattened and fan-shaped, up to 0.5 mm. long, expanding at the time of anthesis to 1.5 mm. diameter and becoming cup-shaped.

Macroscopic features of sori

Sori in inflorescences may be restricted to the floral parts of a minority of spikelets, a condition which can be termed the *Ustilago sphaerogena* form of smut expression (Plate XXIII).

Where sori replace organs of the floret, a spikelet bears a number of globose, green, hispid sori up to 3 mm. diameter. In any infected spikelet there are up to six sori of varying dimensions. When the maximum number of sori is developed one sorus occupies the position of the ovary, three are present in positions where stamens usually are placed, and a further two occur lower down on the axis in the position normally occupied by lodicules (Plate XXIII). There is, however, great variation in this pattern. Not uncommonly fusion of smutted floral organs leads to the formation of a large and somewhat irregular sorus on the apical part of which may be seen remnants of tissue recognizable as styles or stigmas, stamens or lodicules. Sometimes parts of the lemma and palea are incorporated in such a sorus. The sterile floret sometimes is changed, the palea being abnormally hairy at the base. A small hispid structure, the origin of which could not be determined, sometimes develops between the lemma and palea. All sori retain their green colour until maturity, when the soral covering splits irregularly to disclose a dark spore mass in which one or more columellae become visible as the spores are dispersed.

A second sort of smut expression in inflorescences is that which might be termed the *Ustilago crus-galli* form since sori occur in stems and leaves and extend to inflorescences in some plants only. In the latter case almost every spikelet in the inflorescence may be smutted and those not affected are usually infertile. Various components of the spikelets may be affected individually or in combinations to form a large number of irregularly shaped sori. Sporulation in the sterile florets is common, lemmas and paleas being sites for the formation of sori. Phyllody is a characteristic feature. This is partly due to the extension of the distal ends of many sori into flattened green leaf-like structures, but short vegetative shoots also grow from the axis of the inflorescence. Unlike the sori of the *U. sphaerogena* form, these sori do not maintain a regular shape for any one organ of origin and there is considerable disparity between sori. Because of phyllody and the range of plant structures involved it is difficult to identify with certainty the origin of many of the individual sori (Plate xxiv).

Microscopic appearance of sori

The soral covering with layers of host and of fungal origin is a feature common to all sori. The structure of the sori and of the collumellae contained therein varies according to the part of the plant in which sporulation has occurred.

The columella

The term columella is used in reference to the region where the fungus and host tissues are in close contact. The columella in sori developed from organs of the floret is a columnar structure extending upwards from the base of the sorus and ending bluntly in the soral cavity. In sori in vegetative tissues and sometimes in sori in inflorescences (the *U. crus-galli* form) the columella is a pad of tissue with an undulating surface with which hyphae are in close contact. In each case the columella is composed of cells of the host (parenchyma and tracheal elements) and abundant intercellular and intracellular hyphae.

The soral covering

The soral covering consists of two distinct layers, the outer one being tissue of the host plant and the inner layer a fungal sheath (Plate xxiv). The fungal sheath lining the soral cavity is a dense mass of interwoven hyphae which can be separated from the host tissue by gentle manipulation with needles. This sheath is present at an early stage of sporulation and remains virtually unchanged in thickness as the sorus expands and matures. The

TABLE 1
Thickness of layers in the soral covering

Type of sorus	Thickness of host tissue layer (All figures in microns)	Thickness of fungal layer
Fully developed ovary sorus	30-50	30-50
Dehiscing sorus in ovary	10-20	30-40
In stamen	50 (100 at cushions)	10-20
Fused ovary and stamen	10-40	10-20
In lodicule	30-80	10-30

thickness of the host layers and the fungal layers in soral coverings varies, as shown in Table 1. The host tissue has three to five layers of cells which vary in size, ranging in diameter from 10 to 30 μ . In some places large epidermal cells are grouped together to form cushions. It is from cells

of these cushions that most of the hairs of the soral covering arise, although soral hairs occasionally develop from smaller epidermal cells. The hairs are unicellular, elongate, tapering and sharply pointed structures. They vary in length and usually reach 1 to 2 mm. with a basal diameter of 16 to 20 μ . These hairs resemble those found on axes of the inflorescence and are much larger than the hairs found on parts of normal spikelets. While spore formation progresses the soral coverings increase to accommodate the expanding spore mass. As the sorus approaches maturity, cells of the host tissue distort and flatten as they dry and in some areas they may peel off, exposing the fungal sheath which then is the only covering over the spores.

Sori in ovaries

Sori developed from the ovary alone have in most cases a distinct papilla situated on the distal end of the sorus and bearing two styles and stigmas. A light coloured strip extending from this papilla down the anterior side of the sorus can be seen in practically all sori of this type. In this region there are two walls enclosing a flattened, elongate cavity. The outer wall enclosing this cavity consists of three to four layers of parenchymatous cells bounded inside and out by a distinct epidermis. There is profuse hair development on both sides of the outer wall and also on the epidermis of the inner wall.

Dissection showed that remnants of an ovule were often present in this cavity which therefore has been interpreted as being the distorted locule of the ovary. The form and the position of the ovule varied. In some sori it was located in the papillate distal part of a sorus, somewhat removed from the region of sporulation. Its structure then was approximately that of the ovule in a non-infected ovary except that the integuments were densely clothed on both sides with short, hyaline, unicellular hairs. In other sori the ovule appeared to be placed directly on the wall of the sorus and the normal morphological characters of the ovule were no longer evident (Plate xxv, *a*, *b*).

In the early stages of development of sori in ovaries, mycelium sometimes proliferates in the region of ovule attachment. In other cases the mycelium grows into the funicle and may reach the base of the nucellus. The consequent amount and type of distortion appear to be related to the position in the ovary at which massing of mycelium occurs.

Sori in stamens

These sori were up to 2 mm. diameter, globose to flask-shaped, often surmounted by a filament up to 0.5 mm. long and bearing an anther. Such anthers did contain pollen, although it was not determined whether this pollen was fertile.

The first evidence of infection in filaments was a slight swelling and marked hairiness close to the base of the filament. At a later stage a mass of mycelium was found to be present inside the swollen portion. Further growth resulted in the formation of a globose sorus with a typical cylindrical columella inside (Plate xxvi).

Alteration of the staminal filament may extend for some distance above the sorus proper, the remaining length of filament often being distinctly swollen, tapering from sorus to anther and exhibiting marked hairiness.

Sori in lodicules

Sori in lodicules are up to 2 mm. diameter, but even in a single floret there may be one much bigger than the other. The fungal sheath and its

overlying hispid covering are similar to those of sori in other organs. The columella is broadly triangular in section and flattened in the plane of the broad axis of the normal lodicule.

Other sori in inflorescences

The irregular sori of the *Ustilago sphaerogena* form originate in the floral axis, and the regions of sporulation may include basal parts of floral organs, lemmas and paleas. The soral cavity is continuous although invaginations of the soral wall indicate where various host parts have grown together. The columella of these compound sori is broad at the base and divided apically, the points corresponding to the several components of the sorus.

Some sori in inflorescences are not confined to the spikelets and resemble the sori in vegetative parts of the plant. These sori of the *Ustilago crus-galli* form, whether in inflorescences or in vegetative parts, have the soral covering and the columella of the form already described. Other matters of structure are discussed in a later section.

STRUCTURE OF SORI IN VEGETATIVE PARTS

Sori form as irregular blister-like swellings on various vegetative parts of the plant (Plate VII *a, b*). They are usually largest and most numerous, often coalescing, in the nodal regions but occur commonly on the leaf bases, leaf blades and internodes of the stem. The sori, which have green, hispid coverings, vary from smoothly rounded to cerebriform. At nodes and on leaf bases sori may reach a size of 1.0–2.0 cm. diameter. On leaf blades and in internodal regions sori are usually much smaller, 1–several mm. diameter, occurring as isolated blisters or by coalescence forming sori which lie along the leaf or stem. Normal inflorescences are commonly produced by the infected culms. The presence of smut in nodal regions of these culms may stimulate development of axillary buds leading to a rosette of short, leafy axillary shoots emanating from a complex mass of sori. Inflorescences are seldom produced from such shoots. The microscopic structure of the sori is the same as that of galls in inflorescences except for certain details of the form of the columella. The points of difference are mentioned below.

SPORE FORMATION

Kukkonen and Vaissalo (1964) have reviewed the rather limited information that there is on spore formation in smut fungi. Their own electron microscope studies of sporogenesis in *Anthracoidea aspera* confirm earlier work on species of *Ustilago* by De Bary (1887), Lutman (1910) and Hutchins and Lutman (1938) who found that spore initials were embedded in an almost homogeneous gelatinous mass produced by swelling of the sporogenous hyphae. Magnus (1896) and McAlpine (1910) have recorded some details of development of *Ustilago crus-galli*. In that smut gelatinization of hyphae was observed, and weight was given to the supposed basipetal formation of spores in the sporogenous hyphae. The latter observation was the reason for these authors' inclusion of the species in the genus *Cintractia*. De Bary (1887) defined the genus *Sphacelotheca*, emphasising the columella and the fungal sheath surrounding the spore mass as characters by which it was distinguished from *Ustilago*. He said that the development and mature structure of the spore mass of *Sphacelotheca* were the same as those of *Ustilago*. For the latter he described the gelatinization of hyphae synchronously with or even before the basipetal development of the spores.

During the present investigation the modes of spore formation of sori of the *Ustilago sphaerogena* and *U. crus-galli* forms respectively were found to be essentially the same. Sori of the former tend to be symmetrical about the columella while the latter usually have the columella oriented so that the sporulation is above it and not around it. There is a continual production of hyphae at the columella. The walls of these hyphae become gelatinized and hyphal characters disappear. Spore initials then appear in the gelatinous matrix a short distance from the columella, and when first visible are opaque bodies about 3μ in diameter, almost indistinguishable from the rest of the matrix. The spore initials then rapidly expand to a size approaching that of mature spores. Concurrent with the development and expansion of spore initials is the formation of dense echinulations which outline the spores, no spore walls being visible at this stage. Soon after the appearance of echinulations the spore walls become well defined. At first hyaline, they soon become pigmented. As the initials are expanding and maturing, the gelatinous matrix gradually disappears until the spores are connected by thin strands of gelatinous material. In young sori spore masses are agglutinated and possibly the cementing material may be remnants of this matrix. In older sori spores become powdery.

The production of sporogenous mycelium in young sori proceeds at a greater rate than its gelatinization. Thus the columella becomes surrounded by mycelium which is hyaline, thick walled, septate, much branched and $1.0-3.0\mu$ in diameter. It closely resembles the mycelium of the sheath that surrounds the spores.

The gelatinization of sporogenous mycelium that grows around or above the columella tends to be concentrated in a number of discrete regions. The continued growth of mycelium between the regions of rapid gelatinization produces hyphal columns which extend into the developing sorus, the columns being more numerous in the sori in vegetative parts than in floral sori. These columns in which the hyphae lie parallel to one another are associated with crests on the undulating surface of the columella (Plate xxvii). Gelatinization of hyphae of the columns later reduces considerably the extent to which the columns penetrate out into the mature spore mass.

During development of the sorus, spores in all stages of formation can be found. When production of sporogenous mycelium ceases, spore formation continues and mature spores are finally developed very close to the columella.

TAXONOMY

Ustilago tricophora (Link) Kunze. Flora, 13, 369. 1830. *Cacoma tricophorum* Link in Willdenow, Sp. Pl., 6 (2), 3. 1825. *Ustilago sphaerogena* Burrill in Saccardo, Sylloge Fung., 7, 468. 1888. *Ustilago crus-galli* Tracy & Earle, Bull. Torrey bot. Club, 22, 175. 1895. *Ustilago panici-frumentacei* Brefeld, Unters. Gersammt. Mykol., 12, 103. 1895. *Cintractia seymouriana* Magnus, Ber. dt. bot. Ges., 14, 217. 1896. *Cintractia crus-galli* (Tracy & Earle) Magnus, Ber. dt. bot. Ges., 14, 392. 1896. *Ustilago globigena* Speg., Anal. Mus. nac., 6, 208. 1898. *Cintractia sphaerogena* (Burrill) Hume, Proc. Iowa Acad. Sci., 9, 233. 1902. *Ustilago tricophora* (Link) Kunze var. *pacifica* Lavroff, Trudy biol. nauchno-issled. Inst. tomsk. gos. Univ., 2, 9. 1936. *Ustilago tricophora* (Link) Kunze var. *crus-galli* (Tracy & Earle) Lavroff, Trudy biol. nauchno-issled. Inst. tomsk. gos. Univ., 2, 9. 1936.

Sori in organs of the floret, in parts of the inflorescence or in vegetative parts, variable in size and form, with a hispid covering of host tissue. Spore mass agglutinated at first, later pulverulent, surrounded by a fungal sheath

up to 50μ thick. Spores globose, subglobose or ellipsoid, dark, $6-14\mu$ at greatest diameter, ornamented with spines varying in density of distribution, length and shape.

Specimens examined: On *Echinochloa colonum* (L.) Link, Egypt, Ehrenberg, no date (IMI); Sudan, S. A. J. Tarr, 12. x. 1954 (IMI 59760); Louisiana, U.S.A., Atkinson & Forbes, 28. viii. 1936 (BPI); Louisiana, U.S.A., C. R. Ball, 16. viii. 1898 (BPI); Louisiana, U.S.A., I. L. Forbes, 13. ix. 1940 (BPI); Cuba, F. S. Earle, 9. x. 1924 (BPI); Amberley, Qld., R. F. N. Langdon, 29. iii. 1947 (BRIU 415); Lawes, Qld., W. J. Bissett, 28. ii. 1941 (BRIU 501); Nambour, Qld., J. C. Johnson, 26. iii. 1951 (BRIU 270); Cleveland, Qld., O. R. Byrne, 1951 (BRIU 1209); St. Lucia, Qld., R. A. Fullerton, iii. 1966 (BRIU 2283), iii. 1966 (BRIU 2284), iii-iv. 1966 (BRIU 2286), 3. iv. 1966 (BRIU 2287); Meandarra, Qld., R. A. Fullerton, 1. v. 1966 (BRIU 2288); Goondiwindi, Qld., R. A. Fullerton, 2. v. 1966 (BRIU 2289, 2290); Pilliga, N.S.W., J. A. O'Reilly, vi. 1958 (DAR 4863). On *Echinochloa turneriana* Domin., Windorah, Qld., P. J. Skerman, 26. vii. 1946 (BRIU 399), 24. vi. 1949 (BRIU 529). On *Echinochloa holubii* (Stapf) Stapf, Transvaal, Sth. Africa, I. B. Davy, 15. iii. 1912 (PRE 2247, BPI) (TYPE, *Ustilago crus-galli* Tracy & Earle var. *minor* Zundel). On *Echinochloa walteri* (Purch) Heller, Connecticut, U.S.A., G. M. Reid, 14. ix. 1919 (BPI). On *Echinochloa crus-galli* (L.) Beauv., Utah, U.S.A., Tracy & Evans, no. 651, 8. x. 1887 (BPI) (TYPE, *Ustilago crus-galli* Tracy & Earle); Illinois, U.S.A., A. B. Seymour, no. 1892, ix. 1884 (BPI) (TYPE, *Ustilago sphaerogena* Burrill); Argentina, C. Spegazzini, no. 3025, 13. v. 1917 (LPS) (*Ustilago globigena* Speg., det. C. Spegazzini); Nanking, China, F. L. Tai, 3. x. 1929 (BPI); Japan, quarantine interception at Seattle, U.S.A., iii. 1941 (BPI); Mandalay, Burma, A. McKerrel, 17. viii. 1922 (BPI); Arizona, U.S.A., D. F. Cook, 22. ix. 1923 (BPI); California, U.S.A., W. W. Mackie, xii. 1928 (BPI); Colorado, U.S.A., A. S. Hitchcock, 28. viii. 1906 (BPI); Colorado, U.S.A., E. Bartholomew, 4. ix. 1914 (BPI); Connecticut, U.S.A., G. P. Clinton, 22. ix. 1906 (BPI); Connecticut, U.S.A., G. L. Zundel, 30. ix. 1926 (BPI); Florida, U.S.A., H. W. Wedgeworth, 3. vii. 1940 (BPI); Illinois, U.S.A., M. B. Waite, 2. x. 1888 (BPI); Indiana, U.S.A., F. D. Fromme, 1. x. 1913 (BPI); Iowa, U.S.A., L. H. Pammel, 20. ix. 1909 (BPI); Maryland, U.S.A., H. H. McKinney, 25. x. 1944 (BPI); Massachusetts, U.S.A., A. B. Seymour, 10. ix. 1910 (BPI); Minnesota, U.S.A., D. Griffiths, viii. 1896 (BPI); Missouri, U.S.A., J. B. Norton, ix. 1896 (BPI); Nebraska, U.S.A., T. A. Williams, 11. ix. 1890 (BPI); Nevada, U.S.A., O. F. Smith, viii. 1940 (BPI); New Jersey, U.S.A., F. L. Scribner, 24. ix. 1880 (BPI); New Mexico, U.S.A., E. W. D. Holway, 13. ix. 1896 (BPI); New York, U.S.A., R. Latham, 1. x. 1916 (BPI); Oklahoma, U.S.A., W. H. Long, 28. xii. 1909 (BPI); Oregon, U.S.A., J. R. Kienholz, 15. ix. 1937 (BPI); Pennsylvania, U.S.A., G. L. Zundel, 22. ix. 1938 (BPI); Utah, U.S.A., A. D. Garrett, 16. x. 1904 (BPI); Virginia, U.S.A., P. Klaphaak, 12. x. 1922 (BPI); Washington, U.S.A., W. N. Suksdorf, 3. x. 1894 (BPI); Washington, D.C., U.S.A., M. B. Waite, 30. x. 1888 (BPI); Wyoming, U.S.A., A. Nelson, 27. viii. 1904 (BPI); Spain, collector not stated, viii. 1946 (BPI); Morocco, G. Malencon, 9. xi. 1932 (BPI); Nigeria, A. Thompson, 2. iv. 1920 (BPI); Cape Province, South Africa, A. O. D. Mogg, 7. iii. 1934 (PRE 27384; and BPI, as *Ustilago crus-galli* var. *minor* Zundel, det. Zundel); Argentine, T. Rojas, v. 1906 (BPI); New York, U.S.A., R. Latham, x. 1922, and D. Reddick, 25. x. 1941 (BPI, as *Ustilago crus-galli* var. *minor* Zundel, det. Zundel); Transvaal, South Africa, A. O. D. Mogg, 3. vii. 1934 (BPI, as *Ustilago crus-galli* var. *minor* Zundel, det. Zundel); Richmond, N.S.W., W. M. Carne, iii. 1911 (DAR 721), A. Murphy, v. 1961 (DAR 6152); Yenda, N.S.W., collector not stated, iv. 1934

(DAR 1002); Yanco, N.S.W., P. Kable, iii, 1962 (DAR 6981); Baulkham Hills, N.S.W., collector not stated, 1956 (DAR 4862). On *Echinochloa crus-galli* (L.) Beauv. var. *frumentaceae* W. F. Wight, Aspley, Qld., J. G. Morris, iv, 1952 (BRIU 583).

In addition to the specimens already listed, 32 other specimens on *Echinochloa crus-galli* from localities in 14 States of the United States of America were examined. These fungi did not differ significantly from the collections on that grass from the U.S.A. which have been formally listed above.

Notes on specimens examined

(a) Type specimens

Ustilago sphaerogena Burrill. The specimen consists of two smutted spikelets. Floral parts are affected individually. In one spikelet there is a large sorus in the position of the ovary, bearing remnants of the styles on its papillate apex. Three smaller sori surround the large central sorus, occupying the positions of the stamens. Each of these three sori bears an anther on a thin projection from the apex. Two small sori occupy the positions of the lodicules. All sori are covered by hairy tissue of the host. The other spikelet bears several sori occupying the positions of the floral parts. The spores are globose to subglobose, 6–12 μ diameter, densely and sharply echinulate (Plate xxviii).

Ustilago crus-galli Tracy & Earle. The specimen is a small length of stem with a single node and part of a leaf. An axillary shoot is developed from the node. Sori are developed in two hairy leaf galls each about 2.0 mm. diameter. The spores are globose to subglobose, 9.0–13.5 μ diameter, echinulate. The spines are broader at the base (i.e. blunter) and less densely crowded than in *U. sphaerogena* (Plate xxviii).

Ustilago crus-galli Tracy & Earle var. *minor* Zundel. The spores of this specimen are not distinguishable from those of type material of *U. crus-galli*.

(b) Authentic specimens

Ustilago globigena Speg. The specimen is part of an inflorescence with a few smutted spikelets. The sori are developed in floral parts and resemble closely the sori found in the type specimen of *U. sphaerogena*. The spores are not smooth, as stated by Spegazzini (1899), but are echinulate and as suggested by Hirschhorn (1939) are very similar to those of *U. sphaerogena*.

(c) Other specimens

Ustilago tricophora (Link) Kunze. Type material could not be located. By courtesy of the Director of the Commonwealth Mycological Institute we have been able to view a slide made by one of his staff from a smutted specimen of *Echinochloa colonum* which is now in a phanerogamic herbarium (not specified). The specimen had been collected in Egypt by Ehrenberg, who was also the collector of the material from Egypt on which *Ustilago tricophora* is based. There is a strong possibility that Ehrenberg's collection from Egypt went partly to Link and partly to a phanerogamic herbarium, especially as the inflorescence smut of *Echinochloa* affects only a small proportion of the spikelets. The spores of this specimen are globose to subglobose, 9–11 μ diameter and densely echinulate. The spines are slightly coarser and not quite as long as the spines of *Ustilago sphaerogena*. A specimen of smut on *Echinochloa colonum* from the Sudan is very similar to type material of *Ustilago sphaerogena*, as also is smut on *Echinochloa crus-galli* from Cape Province, Union of South Africa. It is believed that

these specimens from Africa, including one in the type host from the type locality of *Ustilago tricophora*, can be accepted as reliable evidence as to the characteristics of *Ustilago tricophora*.

Ustilago sphaerogena Burrill and *U. crus-galli* Tracy & Earle. A majority of the specimens received under the name *Ustilago sphaerogena* bore sori in the floral parts which were the same as those seen in the type specimen of this fungus. The spores also were characteristic of that species. Similarly, most of the specimens which were labelled *Ustilago crus-galli* had the soral and spore characters of the type material of that species. The dimensions of the spores in some specimens varied a little from those of the type specimens of these two species. Specimens of the *U. sphaerogena* form in all except three cases had spores which were within the limits of size of spores of the type specimen, and these exceptions exceeded the upper limit by only one micron. With *U. crus-galli* material the type specimen had spores ranging from 9 to 13.5 μ diameter but in a majority of specimens spores with lower limits down to 6 μ were found. A few specimens included spores which had their longest dimensions one or two microns greater than what were observed in the type specimen. Spores which exhibited these deviations from the limits of size of the type specimen were nearly all markedly subglobose or tending to ellipsoid. For both species the variations in dimensions are not regarded as being of any significance. All specimens include spores with dimensions within the range exhibited by the type specimens.

The type specimen of *Ustilago sphaerogena* exemplifies a form of *Echinochloa* smut where sori are developed in the floral parts alone. The ornamentation of the spores of certain specimens with these symptoms was not typical of *Ustilago sphaerogena*. In some the spines were less densely spaced and the form of the spines tended towards that found in spores of *U. crus-galli*, i.e., somewhat shorter and more broadly based than in *U. sphaerogena*. Three specimens from Louisiana, U.S.A. (collected by Ball in 1898, Atkinson and Forbes in 1936 and Forbes in 1940 respectively) exhibited this intermediate condition of the spines. In specimens from Cuba (coll. Earle, 1924), China (coll. Tai, 1929) and Spain (collector not stated, 1946), the ornamentation of the spores was typical of that found in spores of type material of *U. crus-galli*. A specimen from Nigeria (coll. Thompson, 1920) had spores with the *U. crus-galli* form of spines but with the spines more sparsely placed than in *U. crus-galli*. A few spores of this specimen were smooth. A collection from Oregon, U.S.A., by Kienholz in 1937 which had the *U. sphaerogena* form of sorus had very short, broadly based and sparsely placed spines which could be considered to be within the range of variation of *U. crus-galli*, and indeed this specimen, identified by R. Sprague, came to us under the name *U. crus-galli*.

Two specimens with stem sori typical of *Ustilago crus-galli* (Burma, coll. McKelrel, 1922, and Connecticut, U.S.A., coll. Clinton, 1906) had spores with ornamentation tending towards the form typical of *U. sphaerogena*. The former was labelled *U. sphaerogena*, the latter *U. crus-galli*. Both are intermediate in form between the "typical" conditions of those species.

Specimens of smut on *Echinochloa colonum* from Queensland included some specimens collected in the countryside and others from plants grown in plots at St. Lucia. Most plants from the field bore sori of the *Ustilago sphaerogena* form only and had spores which conformed to those described for *U. sphaerogena*. Exceptions to this were BRIU 2288 which had sori on vegetative parts only, BRIU 2289 which had sori in both vegetative parts and inflorescences, the sori in the latter being of two kinds, the *U. crus-galli* form and the *U. sphaerogena* form, and BRIU 2290 with sori of the *U.*

crus-galli form in both vegetative parts and inflorescences. Plants grown from smut-inoculated seed also included some which bore sori of both the *U. crus-galli* and the *U. sphaerogena* forms (BRIU 2283, 2284, 2286) and one with the *U. crus-galli* form of sorus in vegetative tissues only (BRIU 2287). The ornamentation of the spores in these specimens was variable. In some sori forms of spine were found which were intermediate between the *U. sphaerogena* and *U. crus-galli* forms in width and in distribution on the spore surface. In others, spores of the typical *U. crus-galli* form were found. In essence, the spores and the sori on plants inoculated with spores from the *U. sphaerogena* form of sorus showed variability covering the range of characters which earlier workers had used to delimit two species, namely *U. sphaerogena* and *U. crus-galli*.

Notes on synonymy

Three names have been listed as synonyms of *Ustilago tricophora*, namely *U. panici-frumentacei*, *U. tricophora* var. *pacifica* and *U. tricophora* var. *crus-galli*, although type or authentic specimens have not been seen. Mundkur (1943) has studied *U. panici-frumentacei* and considered its relationship to *U. tricophora*. A small difference in spore size was the only character by which Mundkur could differentiate these two species. Our present studies do not support a concept of species differentiation by size differences of about one micron. Lavroff (1936) included *U. panici-frumentacei* with *U. tricophora*, giving it varietal status in the latter species. He also reduced *U. crus-galli* to the status of a variety of *U. tricophora* (Petraik, 1950).

Mundkur (1943) after examining type material of *U. paradoxa* reported that its spores were entirely smooth. He noted that its mode of germination differs from that of *U. crus-galli* and *U. panici-frumentacei*. In our studies only two specimens, one from Nigeria (BPI) and the other from Queensland (BRIU 2284), have exhibited spores lacking spines. In these fungi the smooth spores were exceptional, the great majority being echinulate. *U. paradoxa* is accepted as being distinct from *U. tricophora* although its habit of attacking only some spikelets in an inflorescence and its hispid soral covering indicate its possible relationship to *U. tricophora*.

DISCUSSION

Several species of *Ustilago* have been described from *Echinochloa* from various parts of the world. One of them, *U. holubii*, destroys the inflorescence and the sorus may extend into the stem below. This characteristic and the smooth to minutely verruculose spores distinguish it from other smuts of these grasses. Common to the rest of the species of *Ustilago* described from *Echinochloa* is a hispid membrane, developed from tissues of the host, covering the fungal structures in the region of sporulation. In a taxonomic study of smuts of this kind we have retained *Ustilago paradoxa*, which has smooth spores, and have grouped the other smuts to make a single species, *Ustilago tricophora*. The description of the latter species has been amended in this paper and it now includes all those smuts of *Echinochloa* which form sori with coverings formed conjointly of fungal tissue and hispid tissue of host origin and which have spores ornamented by spines.

Certain smuts of *Echinochloa* may evoke similar host reactions which are reflected by the uniformity of the host tissues that cover sori and by resemblances between sori on various parts of the plant. Apparent differences in soral structure, e.g. in shape and size, are related to the organ or part

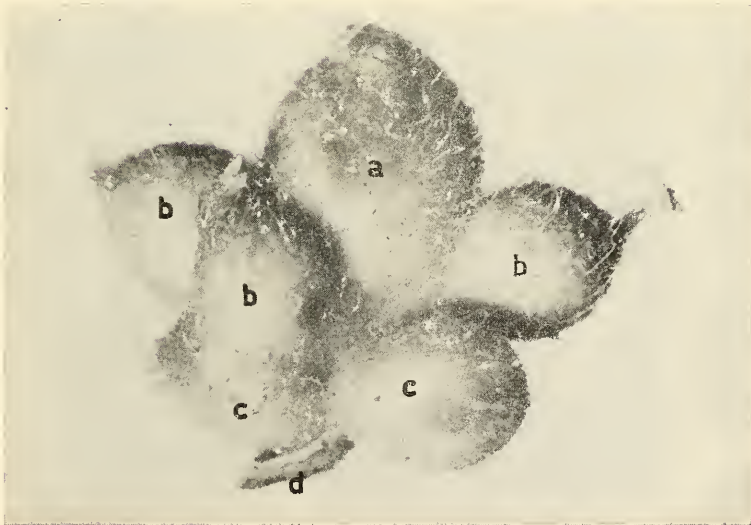
of the plant in which sporulation has occurred. Similarities in soral characters are not in themselves indicative of a taxonomic relationship between smuts. Evidence for such a relationship for the smuts now referred to *Ustilago tricophora* is to be found in the experimental work on inoculation of plants with smut, in patterns of sporogenesis in the different forms of sori, and in comparisons of spore characters of a large number of herbarium specimens.

It has been shown that when *Echinochloa colonum* was inoculated with spores from the *Ustilago sphaerogena* form of sorus individual plants bearing sori of both the *U. sphaerogena* and the *U. crus-galli* forms could be found. In these sori supposedly typical of two different species of smut, there were marked similarities in the development of fungal structures, e.g., columella and fungal sheath, and in sporogenesis. In the inoculated plants as well as in the herbarium specimens, spores representing the intergrades between the *U. sphaerogena* form and the *U. crus-galli* form were found. The extreme cases were the occurrence of the *U. crus-galli* form of spores in sori in the *U. sphaerogena* form of sorus, and vice versa.

Fullerton (1966), who reported on the structure of the sori of the *Ustilago sphaerogena* form of smut of *Echinochloa colonum* suggested that *Sphacelotheca* might be a genus more appropriate than *Ustilago* for the species of smut he studied. The basis for that suggestion was that the smut of *Echinochloa* appeared to have the characteristics of spores enclosed by a fungal sheath and basipetal formation of spores around a columella which in recent years have been accepted as characteristic of the genus *Sphacelotheca* (Fischer, 1953; Fischer and Holton, 1957). Hirschhorn (1939) and McAlpine (1910) have doubted the validity of *Sphacelotheca* as a genus distinct from *Ustilago*. McAlpine noted that a columella occurs in other smuts such as species of *Cintractia* and *Sorosporium*, and that there are ". . . various gradations in the formation of a fungus membrane enclosing the spores . . .". He indicated that there were difficulties in deciding whether a sheath should be regarded as evanescent or persistent. Concerning the other characters for distinguishing *Sphacelotheca* from *Ustilago*, namely the basipetal formation of spores in the former which is lacking in the latter, there seems to be no grounds for accepting such a distinction. De Bary (1887) declared that in the smut on which *Sphacelotheca* was based, namely *Ustilago hydropiperis*, ". . . the development and mature structure of the spore-mass are the same as those of *Ustilago* . . .". In the sections cut from *Ustilago tricophora* in various stages of development we have noted that for some time there is production of sporogenous hyphae around the columella. Spores develop from the first-formed hyphae and are pushed outwards as more sporogenous hyphae grow. Spore formation is a continuous process that occurs progressively while sporogenous hyphae are forming rather than being the basipetal formation of spores from a mass of pre-formed hyphae, progressing from the region of the fungal sheath towards the columella. The basipetal formation of spores often quoted as a characteristic of the genus *Sphacelotheca* may be more apparent than real. Further studies of sporogenesis in smut fungi which at present are included in *Sphacelotheca* are in progress. For the present no change in the generic position of the species *tricophora* will be made.

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Top.—*Echinochloa colonum* with *Ustilago sphaerogena* form of smut.
Bottom.—Sori developed in floral parts of *Echinochloa colonum*.